Appln. No.: 10/672,225

Amendment Dated March 13, 2008 Reply to Office Action of July 11, 2007

<u>Amendments to the Claims:</u> This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Previously Presented) A method of determining a binding capacity of a surface, the method comprising:

providing the surface, said surface comprising a first reactive moiety;

contacting the surface with a fluorophore comprising a fluorescent moiety and a second reactive moiety, thereby causing a reaction between the first and second reactive moieties and forming a linking bond or group that binds the fluorescent moiety to the surface;

cleaving the linking bond or group, thereby liberating the fluorescent moiety from the surface;

exposing the liberated fluorescent moiety to exciting radiation;

measuring a signal emitted by the liberated fluorescent moiety; and

calculating the binding capacity of the surface from the strength of the signal.

- 2. (Previously Presented) The method of claim 1, wherein the linking bond or group comprises a disulfide bond or an aromatic azo bond and wherein the step of cleaving the linking bond or group comprises cleaving the disulfide or aromatic azo bond.
- 3. (Previously Presented) The method of claim 2, wherein the linking bond or group comprises a disulfide bond.
- 4. (Withdrawn Currently Amended) The method of claim 2, wherein the linking bond or group comprises an aromatic azo group represented by the formula:

$$-R^2-N=N-$$

wherein R<sup>2</sup> is a <del>divalent aromatic</del> moiety selected from the group consisting of a heterocyclic groups and electron-deficient aromatic groups.

5. (Currently Amended) The method of claim 2, wherein the fluorophore is a thiol-containing fluorescent compound-represented by the formula:

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wherein FI comprises the fluorescent moiety, and wherein FI-SH is selected from the group consisting of L-cysteine derivatives bearing fluorescent substituents and compounds wherein FI the fluorescent moiety comprises a fluorescein moiety.

6. (Previously Presented) The method of claim 5, wherein the thiol-containing fluorescent compound is selected from the group consisting of:

7. (Previously Presented) The method of claim 5, wherein the thiol-containing fluorescent compound is

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8. (Withdrawn - Previously Presented) The method of claim 5, wherein the thiol-containing fluorescent compound is:

9. (Withdrawn - Previously Presented) The method of claim 2, wherein the fluorophore is a thiol-reactive fluorescent compound represented by the formula:

wherein X is selected from the group consisting of Cl,  $SO_3(C_1-C_6 \text{ alkyl})$ , and  $S-R^2$ , wherein  $R^2$  is a heterocyclic group or an electron-deficient aromatic group.

- 10. (Withdrawn Previously Presented) The method of claim 9, wherein R<sup>2</sup> is a pyridyl group or a phenyl group substituted with one or more electron-withdrawing substituents.
- 11. (Withdrawn Previously Presented) The method of claim 9, wherein the thiol-reactive fluorescent compound is selected from the group consisting of:

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- 12. (Withdrawn Previously Presented) The method of claim 2, wherein the second reactive moiety is bound to the fluorescent moiety by the disulfide bond or aromatic azo bond.
- 13. (Withdrawn Previously Presented) The method of claim 12, wherein the second reactive moiety is selected from the group consisting of an amino group, a thiol group, a protected thiol group, and an epoxy group.
- 14. (Previously Presented) The method of claim 2, wherein the surface is selected from the group consisting of a polymer, a metal, a biomaterial, a ceramic, and a semiconductor.
- 15. (Withdrawn Previously Presented) The method of claim 14, wherein the surface is polyurethane.
- 16. (Previously Presented) The method of claim 2, wherein the first reactive moiety is a thiol, a thiol-reactive group or a group adapted to be converted into a thiol or a thiol-reactive group.
- 17. (Withdrawn Previously Presented) The method of claim 2, wherein the first reactive moiety is a thiol group or an amino group.
- 18. (Withdrawn Previously Presented) The method of claim 2, wherein the first reactive moiety is a reaction product of a surface thiol group or surface amino group with 5,5'-dithio-bis(2-nitrobenzoic acid) or succinimidyl 3-(2-pyridyldithio)propionate.
- 19. (Previously Presented) The method of claim 2, wherein the first reactive moiety is a dithio group.
- 20. (Previously Presented) The method of claim 2, wherein the disulfide bond or aromatic azo bond is cleaved by using a reducing agent selected from the group consisting of dithiothreitol,  $\beta$ -mercaptoethanol, mercaptoethylamine hydrochloride, a borohydride, and a phosphine.
- 21. (Withdrawn) The method of claim 20, wherein the borohydride is sodium borohydride.
- 22. (Previously Presented) The method of claim 20, wherein the phosphine is selected from the group consisting of tris(2-cyanoethyl)phosphine, tris(2-carboxyethyl)phosphine and trimethylphosphine.
- 23. (Withdrawn) A kit for practicing of method of claim 2, the kit comprising a fluorophore.

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- 24. (Withdrawn) The kit of claim 23, wherein the fluorophore comprises the fluorescent moiety and a linking bond precursor.
- 25. (Withdrawn) The kit of claim 23, wherein the linking bond precursor is adapted to form a cleavable disulfide bond or an aromatic azo group.
  - 26. (Withdrawn) The kit of claim 25, wherein the linking bond precursor is —SH.
- 27. (Withdrawn Currently Amended) The kit of claim 25, wherein the linking bond precursor is represented by a formula:

$$-S-X$$

wherein X is a member selected from the group consisting of Cl,  $SO_3(C_1-C_6 \text{ alkyl})$ , and  $S-R^2$ , wherein  $R^2$  is a heterocyclic group or an electron-deficient aromatic group.

- 28. (Withdrawn) The kit of claim 23, wherein the fluorophore further comprises a functional group, wherein the functional group is bound to the fluorescent moiety by the cleavable bond and is adapted to react with the reactive moiety to form an uncleavable bond.
- 29. (Withdrawn) The kit of claim 28, wherein the functional group is a member selected from the group consisting of an amino group, a thiol group, a protected thiol group, and an epoxy group.
  - 30. (Withdrawn) The kit of claim 28, wherein the uncleavable bond is an amide bond.
- 31. (New) The method of claim 1, further comprising, after contacting the surface with the fluorophore and before cleaving the linking bond or group, a step of washing the surface to remove unbound fluorophore.